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Literature Review

Beetroot Juice and Exercise Performance

Abstract

Beetroot juice (BR) has gained considerable research interest for its ergogenic effects in regards to improving exercise and sports performance. This increased interest is due to BR, which is rich in inorganic nitrate (NO_3^-), providing an alternative pathway for increasing the pools of bioavailable nitric oxide (NO) in the body. The literature has investigated the effects of BR in a variety of exercise and sporting scenarios. Of such, the vast majority has focused on trained populations in aerobic scenarios with chronic and acute dose protocols, while other research has focused on recreationally active populations. The outcomes of the research have not always been consistent, with many studies demonstrating significant ergogenic benefits of BR and others unable to provide favourable outcomes. It is clear from the research reviewed that BR can be used as an ergogenic aid in recreationally active, trained and clinical subjects. Although not all studies agree, performance outcomes range from reduced time to completion (TTC) and oxygen uptake ($\dot{V}O_2$) and increased power output (PO) and exercise tolerance, all of which have been demonstrated in well controlled study designs. Research is vast, particularly in trained populations, with this leading to a need for further research in recreationally active populations. In addition, the overwhelming majority of these studies have been conducted on males, both recreationally and trained, which opens up a requirement for specific female focused studies within the literature.

Introduction

Beetroot juice (BR) has gained considerable research interest for its ergogenic effects in regards to improving exercise and sports performance (Hoon et al., 2013). This increased interest is due to BR, which is rich in inorganic nitrate (NO_3^-), providing an alternative pathway for increasing the pools of bioavailable nitric oxide (NO) in the body, as opposed to the classically thought of L-arginine-NO pathway (Lundberg, Weitzberg & Gladwin, 2008). Through this alternate pathway, NO is facilitated through BR via the reduction of NO_3^- to nitrite (NO_2^-) and subsequently to NO, also known as the nitrate-nitrite-NO pathway (Bailey et al., 2012; Lundberg & Govoni, 2004). Numerous physiological pathways have been shown to be mediated by NO including the vasodilation of blood vessels (Förstermann & Sessa, 2012), glucose uptake (Tanaka et al., 2003), muscle contraction (Linden et al., 2011) and neurotransmission (Vincent, 2010), all of which contribute to the growing interest in the ergogenic effects of NO (Bescós et al., 2012a).

The literature has investigated the effects of BR in a variety of exercise and sporting scenarios. Of such, the vast majority has focused on trained populations in aerobic scenarios with chronic dose (Bond, Morton & Braakhuis, 2012; Cermak, Gibala & van Loon, 2012; Christensen, Nyberg & Bangsbo, 2013; Larsen et al., 2007; Wilkerson et al., 2012) and acute dose protocols (Cermak et al., 2012; Hoon et al., 2014; Lansley et al., 2011b; Muggeridge et al., 2013). However, research has also been conducted in recreationally active populations but is not as vast (Bailey et al., 2009; Kelly et al., 2013; Kenjale et al., 2011; Lansley et al., 2011a; Vanhatalo et al., 2010; Wylie et al., 2013) and

there is currently limited research on resistance training protocols (Bailey et al., 2010). The outcomes of the research have not always been consistent, with many studies demonstrating significant ergogenic benefits of BR (Bailey et al., 2009; Bailey et al., 2010; Bond, Morton & Braakhuis, 2012; Cermak, Gibala & van Loon, 2012; Hoon et al., 2014; Kelly et al., 2013; Lansley et al., 2011a; Lansley et al., 2011b; Larsen et al., 2007; Vanhatalo et al., 2010) and others unable to provide favourable outcomes (Cermak et al., 2012; Christensen, Nyberg & Bangsbo, 2013; Muggeridge et al., 2013; Wilkerson et al., 2012). These mixed findings can be due, in part, to the variety in methodical procedures being implemented, ranging from subject training status, duration and quantity of BR dose and the type of energy systems used during the respective exercise measurement procedures. The aim of this review is to provide a broad-based consideration of the literature investigating the ergogenic effects of BR on different populations with the use of varying modes, durations and intensities of exercise.

The studies included within this review were obtained from the data bases SPORTDiscus, PubMed, ScienceDirect, MEDLINE, Web of Science. Academic oneFile and Google Scholar. The key terms used were beetroot, nitrate, ergogenic and performance. Studies were excluded if they used additional factors in conjunction with BR such as caffeine or sodium bicarbonate and/or did not investigate exercise or sports performance.

Beetroot Juice and Exercise Performance

Acute Dose with Aerobic Exercise in Trained Populations

Hoon et al. (2014), using a placebo-controlled, double-blind crossover design, investigated the effects of a 70ml placebo (PLAC; NO_3^- depleted BR), single dose (4.2 mmol NO_3^-) and double dose of BR (8.4 mmol NO_3^-) on 2000m rowing ergometer time trial (TT) in 10 highly trained male rowers who ingested the BR two hours before commencement of the TT. A post-only crossover spreadsheet (Hopkins, 2007) was used which reduces the possibility for cofounding effects when age and fitness levels may be unequal in experimental groups (Batterham & Cox, 2006). The crossover spreadsheet showed baseline plasma NO_3^- (1.0 ± 6.8 pM) and NO_2^- (-23.9 ± 17.5 nM) were elevated following the single dose (99.2 ± 12.7 pM, almost certainly positive; 54.9 ± 61.8 nM, very likely positive) and double dose of BR (180.6 ± 46.2 pM, almost certainly positive; 83.3 ± 80.7 nM, very likely positive). Compared to PLAC 2000m time to completion (TTC) (06:23.5 ± 9.0 s), the single dose of BR had a likely trivial effect (06:23.4 ± 8.7 s) whereas the double dose showed a possible beneficial effect on performance (06:21.9 ± 9.0 s). The authors suggest the possible beneficial improvement, only residing from the double dose of BR, can be attributed to the high training level of the subjects whose baseline NO_3^- levels may have been elevated compared to recreationally active populations as shown by a 28% elevation (45 ± 2 vs. 34 ± 2 µM, $p < .01$) in research by Jungersten et al. (1997) with an even greater increase of 96% found by Schena et al. (2002) (49 ± 17 vs. 25 ± 2 µM, $p < .01$), in addition to the cardiovascular system nearing physiological capacity (Bescós et al., 2012a). This finding supports the requirement of a higher intake of NO_3^- to elicit a performance improvement in trained populations. Furthermore,

Lundberg, Weitzberg & Gladwin (2008) have shown the reduction of $N0_2^-$ to N0 (subsequent pathway from $N0_3^-$) to be more active in acidic environments, which would have been prominent during the 2000m ergometer row, compared to longer durations of aerobic exercise whereby the utilisation of oxygen (O_2) is greater (Jones & Carter, 2000) potentially explaining why a BR related improvement was found.

Muggeridge et al. (2013), using eight trained, male, flatwater kayakers, showed similar findings to Hoon et al. (2014) whereby an acute dose of BR (~5 mmol $N0_3^-$) compared to PLAC (tomato juice, negligible $N0_3^-$) induced no effect on performance. The respective beverages were ingested three hours before kayak ergometer performance trials consisting of 15 min paddling at 60% maximum work rate, five 10s sprints and a 1km TT. Mean oxygen consumption ($\dot{V}O_2$) during the 15min steady state trial was 3.1% lower with BR compared to PLAC (p = .010) and also lower in the 1km TT (95% confidence interval (CI) 0.5-2.8 $ml \cdot kg^{-1} \cdot min^{-1}$, p = .014). However, this had no significant effect on mean power output (PO) during the steady state trial between BR (108 ± 23 W) and PLAC (108 ± 22 W, p = .428) and peak PO during the 10s sprints (p = .590). There was no significant difference shown in TT completion in BR (276 ± 5 s) compared to PLAC (277 ± 5 s, p = .539). Although $\dot{V}O_2$ was reduced, this did not carry over into any performance benefits which supports the findings from Hoon et al. (2014) in which an acute dose of BR may not be substantial to provide favourable performance outcomes in trained populations, with a higher dose likely to be required. Furthermore, the authors state the individual variability in response to $N0_3^-$ ingestion, due to varying levels of baseline $N0_2^-$ levels in the subjects, may have contributed to the findings.

Lansley et al. (2011b), using nine competitive male cyclists, investigated the effects of an acute dose of BR on PO, peak oxygen consumption ($\dot{V}O_{2peak}$) and cycling TT which was administered using a placebo-controlled, randomised, double-blind crossover design. Subjects consumed either 0.5L BR (6.2 mmol NO_3^-) or a PLAC (NO_3^- depleted BR) two and a half hours before a 4km and 16km cycling TT. Following the consumption of BR, plasma NO_2^- increased by 138% and TT TTC and PO increased in both 4km (2.8% and 5% respectively, p <.01) and 16km TT (2.7% and 6% respectively, p <.01) in comparison to the PLAC. During the 4km and 16km TT, $\dot{V}O_{2peak}$ was not significantly different in BR (4.46 ± 0.50 and 4.23 ± 0.47 L·min^{-1} respectively) compared with PLAC (4.36 ± 0.47 and 4.19 ± 0.56 L·min^{-1} respectively). Although a marginally smaller dose of NO_3^- was used (6.2 mmol NO_3^-), these findings support the acute double dose of BR (8.4 mmol NO_3^-) implemented by Hoon et al. (2014) in improving TTC in respective athletic disciplines within trained populations. However, the authors did highlight limitations with regard to subject adherence to dietary protocols (TT were rescheduled four times due to poor dietary adherence, only if reported by the subject). In addition, hydration and sleep quality were not measured which could not be ruled out in affecting the results. This study also complements the findings of Cermak, Gibala & van Loon (2012), although a chronic dose study, by showing NO_3^- supplementation provides ergogenic benefits during a variety of short and longer durations of exercise in the research by Lansley et al. (2011b) (~6.3 min and ~75 min, 2.8% and 2.7% TT improvement respectively) and Cermak, Gibala & van Loon (2012) (~15.8 min, 1.2% TT improvement). The improvement contrast in these respective studies (2.8% and 2.7% vs. 1.2%) can be attributed to the marginal difference in training status of the subjects which may have reduced the

ergogenic effects of BR (Bescós et al., 2012a). Furthermore, Cermak, Gibala & van Loon (2012) used a 60 min submaximal protocol immediately preceding their TT which may have lowered the effects of the BR as NO_3^- supplementation has been shown to be more effective during shorter bouts of exercise (Lundberg, Weitzberg & Gladwin, 2008).

The research protocol implemented by Lansley et al. (2011b) is similar in design to that of Cermak et al. (2012) who, when supplementing 20 trained male cyclists, was unable to show that an acute dose of 140ml BR (8.7 mmol NO_3^-) compared to a PLAC (NO_3^- depleted BR) two and a half hours before a one hour cycling TT provided any benefit on TTC (BR: 65.5 ± 1.1 vs. PLAC: 65 ± 1.1 s, p >.05), PO (BR: 275 ± 7 vs. PLAC: 278 ± 7 W, p >.05) and HR (BR: 170 ± 2 vs. PLAC: 170 ± 2 beats/min, p >.05). The contrast in findings between these two studies can potentially be explained by the difference in achieved cycling intensity and/or duration during the respective TT which has been shown to be an important factor in the reduction of NO_2^- to NO (Wilkerson et al., 2012). Cermak et al. (2012) had subjects cycle at 69% ± 1% of their respective W_{max} which required subjects 65 ± 1 min to complete the TT. In contrast, although Lansley et al. (2011b) did not record subjects W_{max}, they were required to complete shorter TT (6-27min) and therefore it would be plausible to consider the subjects completed the TT with a higher intensity resulting in greater NO production from the more acidic (Lundberg, Weitzberg & Gladwin, 2008; Modin et al., 2001) and hypoxic environment (Castello et al., 2006; Cosby et al., 2003). Wilkerson et al. (2012) further supports the significance of intensity and/or duration of exercise relating to the production of NO following NO_3^- supplementation with no significant difference found in TTC (BR: 136.7 ± 5.6 vs. PLAC:

137.9 ± 6.4 min, p >.05) and PO (BR: 238 ± 22 vs. PLAC: 235 ± 27 W, p >.05) after an acute dose of 0.5L BR (6.2 mmol NO_3^-) or 0.5L PLAC (NO_3^- depleted BR) two and a half hours preceding a 50 mile cycling TT in eight well trained male cyclists. However, BR was shown to lower mean $\dot{V}O_2$ by 1.8% which although not significant (p = .06), resulted in a significant increase in PO/$\dot{V}O_2$ ratio by 3% (BR: 67.4 ± 5.5 vs. PLAC: 65.3 ± 4.8 W L·min^{-1}, p <.05). The reduction in submaximal $\dot{V}O_2$ is consistent with the 3.5% and 5.1% respective reductions in submaximal $\dot{V}O_2$ found by Cermak, Gibala & van Loon (2012). Larsen et al. (2007) also found a reduction in submaximal $\dot{V}O_2$ when using dietary NO_3^- supplementation from 2.98 ± 0.58 L min^{-1} to 2.82 ± 0.58 L min^{-1} (p <.02) which resulted in the efficiency of cycling in nine well trained males improving by 1.4% (BR: 21.1 ± 1.3 vs. PLAC: 19.7 ± 1.6%, p <.01).

Acute doses of BR are shown to elicit mixed responses in exercise performance in trained populations. This seems to be dependent on the intensity of exercise and the training level of the subjects as to the performance outcome with doses >4 mmol NO_3^- potentially providing more positive outcomes. Chronic BR dose studies have also been investigated extensively on trained populations.

Chronic Dose with Aerobic Exercise in Trained Populations

Bond, Morton & Braakhuis (2012) used a placebo-controlled, double-blind crossover design with a chronic dose of 0.5L BR (5.5 mmol/day NO_3^-) or PLAC (blackcurrant juice, negligible NO_3^-) over six days on 14 well trained junior rowers. On the sixth day of

supplementation, following the last dose of BR or PLAC, subjects performed six 500m rowing ergometer intervals at maximum intensity with 90s recovery between intervals. Across all intervals, TTC in BR was likely improved compared with PLAC (0.4, 95% CI ± 1.0%). TTC in intervals 1-3 revealed a possible negative effect of BR (1.0, 95% CI ± 1.7%) whereas intervals 4-6 showed an almost certain benefit (1.7, 95% CI ± 1.0%). However, there were no significant differences in diastolic blood pressure, heart rate (HR), oxygen saturation, lactate accumulation or urine pH between the BR and PLAC. The improvement found in intervals 4-6 supports the finding of Lundberg, Weitzberg & Gladwin (2008) and Hoon et al. (2014) whereby an acidic environment provides a greater reduction of $N0_2^-$ to N0 which would have been prominent during the 500m intervals. The O_2 deficit may have been greater towards the last half of the trial which may explain the negligible effects of BR from intervals 1-3. The marginal improvements BR showed to elicit on intervals 1-3 (1.7%) may be viewed as insignificant, however, in the context of elite competition, a marginal performance improvement of at least 0.6% can be of great significance (Paton & Hopkins, 2006). In agreement, the research previously mentioned by Cermak, Gibala & van Loon (2012) revealed consistent findings with 12 trained male cyclists or triathletes when using a chronic dose of 140 ml/day of BR (~8 mmol/day $N0_3^-$) over six days compared with a PLAC ($N0_3^-$ depleted BR). Following the sixth day of supplementation, subjects completed 60 min of submaximal cycling (2 × 30 min at 45% and 65% W_{max}) followed by a 10 km TT. It was shown that BR improved TT performance by 1.2% (BR: 953 ± 18 vs. PLAC: 965 ± 18 s, $p < .005$), PO by 2.1% (BR: 294 ± 12 vs. PLAC: 288 ± 12 W, $p < .05$) and lowered submaximal $\dot{V}O_2$ by 3.5% at 45% W_{max} (BR: 1.92 ± 0.06 vs. PLAC: 2.02 ± 0.09 L/min, $p < .05$) and 5.1% at 65% W_{max} (BR: 2.94 ±

0.12 vs. PLAC: 3.11 ± 0.12 L/min, p <.05). The reduction in submaximal $\dot{V}O_2$ was viewed as surprising as this has been shown to have minimal fluctuations for any individual, at any given work rate, regardless of training status, diet or age (Moseley et al., 2004). The reduction in $\dot{V}O_2$ was attributed to the roles $N0_2^-$ and NO exhibit in cellular O_2 utilization (Clementi & Nisoli, 2005; Dejam et al., 2004).

Contrasting findings were observed in a study by Christensen, Nyberg & Bangsbo (2013) whereby 10 elite male cyclists, with an average maximal oxygen consumption ($\dot{V}O_{2max}$) of 72 ± 4 $ml \cdot kg^{-1} \cdot min^{-1}$ underwent a 400 kcal cycling TT and PO comparisons during six 20s sprints with 100s recovery between intervals. The subjects consumed 0.5L of BR (6.2 mmol $N0_3^-$) or PLAC (0.5L blackcurrant juice, negligible $N0_3^-$) for six days in a placebo-controlled, randomised, double-blind, crossover design. Plasma $N0_2^-$ was higher in BR (159 ± 103 µM) compared with PLAC (40 ± 7 µM, p < .01) after six days of respective beverage intake. Despite the elevation in plasma $N0_2^-$, there were similarities found with TT performance in BR (18:20 min/s) compared with PLAC (18:37 min/s) and average sprint PO (BR: 290 ± 43 vs. PLAC: 285 ± 44 W). This finding highlights BR may elicit a positive effect on performance in moderately trained subjects (Bond, Morton & Braakhuis, 2012; Lansley et al., 2011b), however, in elite subjects, a similar BR dosage (5.5-6.2 mmol $N0_3^-$) may not be sufficient to provide a performance benefit and may suggest the need for a higher dose in this specific population due to the increased development of the cardiorespiratory system (Bescós et al., 2012a).

There appears to be greater benefit with chronic doses of BR in trained populations compared to acute protocols primarily from the accumulation of $N0_3^-$ providing an

ergogenic effect, while more elite athletes may have a blunted response to BR. The research on BR and recreationally active populations is not as extensive.

Chronic Dose with Aerobic Exercise in Recreationally Active Populations

Lansley et al. (2011a) investigated the effects of BR on nine physically active males who consumed either 0.5L BR (6.2 mmol/day NO_3^-) or PLAC (0.5L NO_3^- depleted BR) for six days prior to submaximal and severe intensity bouts of "step" treadmill running. In agreement with trained population research (Cermak, Gibala & van Loon, 2012; Larsen et al., 2007; Wilkerson et al., 2012) BR reduced the end $\dot{V}O_2$ cost by 7% compared with PLAC during both submaximal (PLAC: 2.26 ± 0.27 vs. BR: 2.10 ± 0.28 L min^{-1}, $p < .01$) and high intensity running (PLAC: 3.77 ± 0.57 vs. BR: 3.50 ± 0.62 L min^{-1}, $p < .01$). Furthermore, BR increased time to exhaustion during high intensity running by 15% compared to PLAC (PLAC: 7.6 ± 1.5 vs. BR: 8.7 ± 1.8 min, $p < .01$). The authors proposed the ergogenic benefits found in the study were due to the high NO_3^- content in BR which can have meaningful implications on daily tasks in elderly and clinical populations as a result of the reduced $\dot{V}O_2$ observed in submaximal exercise.

In similar findings to Lansley et al. (2011a), an improvement in exercise tolerance was also found by Kelly et al. (2013) during four separate ramp incremental tests on a cycle ergometer. Nine recreationally active males were supplemented with either 0.5L BR (8.2 mmol/day NO_3^-) or 0.5L/day PLAC (NO_3^- depleted BR) for a minimum of seven, and up to a maximum of 12 days. Exercise tolerance was significantly improved through the ingestion of BR by 17% at 60% (BR: 696 ± 120 vs. PLAC: 593 ± 68 s, $p < .05$), 16% at 70% (BR: 452 ± 106 vs. PLAC: 390 ± 86 s, $p < .05$), 12% at 80% (BR: 294 ± 50 vs. PLAC: 263 ± 50

s, p <.05) and 10% at 100% peak power, however this particular result was not significant (BR: 182 ± 37 vs. PLAC: 166 ± 26 s, p =.10). Although increased exercise tolerance was found at higher intensities of peak power, the greater improvements were found at lower percentages which conflicts with research demonstrating NO_3^- supplementation shows greater effectiveness during shorter, more acidic exercise bouts (Lundberg, Weitzberg & Gladwin, 2008; Modin et al., 2001).

Bailey et al. (2009), using a double-blind crossover design, showed similar findings to Lansley et al. (2011a) with a reduction observed in the $\dot{V}O_2$ cost of exercise when supplementing eight healthy, recreationally active males with either 0.5L of BR (5.5 mmol/day NO_3^-) or PLAC (blackcurrant cordial, negligible NO_3^-) for six days. Progressive "step" cycling protocols were performed at moderate (80% gas exchange threshold; GET) and severe intensity (70% difference between PO at GET and $\dot{V}O_{2peak}$) until the subjects were unable to continue. The ingestion of BR reduced the primary amplitude of $\dot{V}O_2$ by 19% in moderate intensity cycling (PLAC: 640 ± 146 vs. BR: 521 ± 153 ml·min^{-1}, p <.01) with this, in contrast, shown to be increased by 7% during high intensity cycling (PLAC: 2,158 ± 168 vs. BR: 2,345 ± 179 ml·min^{-1} , p < .05) which is commonly observed during initial phases of high intensity exercise (Burnley et al., 2000; Jones, Koppo & Burnley, 2003). The subsequent high intensity $\dot{V}O_2$ slow component was reduced by 23% (PLAC: 739± 242 vs. BR: 568 ± 195 ml·min^{-1}, p < .05) although these findings may not relate to a realistic scenario as the high intensity exercise was performed directly after the moderate intensity protocol. The authors concluded that NO_3^- supplementation has the potential to improve exercise tolerance, especially during high intensity exercise.

Wylie et al. (2013) also agrees with the reduced $\dot{V}O_2$ cost in recreationally active populations as shown by a 2% (BR: 140: 1.64 ± 0.23 vs. PLAC: 140: 1.67 ± 0.21 L·min^{-1}, *p* = .06) and 3% (BR: 280:1.60 ± 0.23 vs. PLAC: 280:1.65 ± 0.19 L·min^{-1} , *p* <.05) reduction in submaximal $\dot{V}O_2$ during cycling following five days of 140ml (8.4 mmol NO_3^-) and 280ml (16.8 mmol NO_3^-) of BR in 10 healthy males.

The minimal time required to elicit a performance benefit on exercise after ingesting BR has yet to be confirmed, although, one attempt to answer this was by Vanhatalo et al. (2010) who examined acute (one and five days) and chronic (15 days) BR supplementation (0.5L, 5.2 mmol/day NO_3^-) compared to PLAC (blackcurrant cordial, negligible NO_3^-) on a moderate intensity step test (90% GET) and incremental cycle ergometer ramp test (work rate increase one W every two seconds until exhaustion) in eight healthy subjects. End exercise $\dot{V}O_2$ consumption was lower during the moderate intensity step test after one day of BR (BR: 1.35 ± 0.17 vs. PLAC: 1.40 ± 0.20 L·min^{-1}, *p* <.05) and was consistent throughout five (BR: 1.38 ± 0.20 vs. PLAC: 1.45 ± 0.21 L·min^{-1} *p* <.05) and 15 days (BR: 1.37 ± 0.23 vs. PLAC: 1.43 ± 0.23 L·min^{-1}, *p* <.05). However, at day 15, $\dot{V}O_{2max}$ (BR: 3.50 ± 0.82 vs. PLAC: 3.42 ± 0.88 L·min^{-1}) and PO (BR: 331 ± 68 vs. PLAC: 323 ± 68 W) were both higher compared with one day of BR supplementation (3.42 ± 0.82 L·min^{-1} and 325 ± 71 W, both *p* <.05). This contributes to the support of a greater performance benefit from chronic BR supplementation showing consistency with Lansley et al. (2011a) and Bailey et al. (2009).

It is clear that chronic dose BR protocols in recreationally active populations are successful, primarily through reducing the $\dot{V}O_2$ cost of exercise. However, research using acute BR doses on recreational subjects has yet to be thoroughly investigated.

Acute Dose with Aerobic Exercise in a Clinical Setting

Acute doses of BR in recreationally active populations are limited. One such study, Kenjale et al. (2011), although not focused on recreationally active individuals, gave a specific implication in a clinical setting on the effects of BR and subjects with peripheral arterial disease (PAD). This study follows up from Lansley et al. (2011a) whereby BR was suggested to be of benefit in clinical scenarios. Eight subjects with diagnosed PAD consumed 0.5L BR (18.1 mmol $N0_3^-$) or PLAC (0.5L orange juice) three hours prior to a maximal treadmill cardiopulmonary exercise (CPX) test. Near-infrared spectroscopy was used to measure gastrocnemius oxygenation during the CPX. Compared to PLAC, BR allowed subjects to walk 18% longer before the onset of claudication pain (BR: 215 ± 99 vs. PLAC: 183 ± 84 s, $p < .01$) and maintain total peak walking time by 17% (BR: 533 ± 233 vs. PLAC: 467 ± 223 s, $p < .05$). Fractional O_2 extraction of the gastrocnemius during the CPX was also lowered by BR (BR: 7.3 ± 6.2 vs. PLAC: 10.4 ± 6.1 arbitrary units, $p < .01$). Although the $N0_3^-$ dose used was 12 times greater than the average daily dietary intake of $N0_3^-$ (Mesinga, Speijers & Meulenbelt, 2003), the results show BR increases NO signalling which results in improved exercise tolerance and peripheral tissue oxygenation in individuals with PAD.

Another area which has received limited research is the effects of BR on resistance exercise.

Resistance Exercise

With specific regard to resistance exercise, Bailey et al. (2010) recruited seven recreationally active males in a randomised, double-blind crossover study. The purpose was to investigate the effects of chronic BR supplementation on 15% and 30% maximal voluntary isometric contractions during "step" knee extension tests to determine the muscle metabolic response, utilising phosphorus magnetic resonance spectroscopy (P-MRS) (Grassi et al., 2003), and $\dot{V}O_2$ response to exercise. Subjects consumed either 0.5L of BR (5.1 mmol/day $N0_3^-$) or 0.5L PLAC (blackcurrant juice cordial, negligible $N0_3^-$) for six days with the knee extension tests taking place on the last three days of supplementation. During 15% isometric contractions, the reduction in muscle phosphocreatine concentration (PC_r) was attenuated by BR (5.2 ± 0.8 mM) compared to PLAC (8.1 ± 1.2 mM, p <.05) and decreased pulmonary $\dot{V}O_2$ by 25% (BR: 362 ± 30 vs. PLAC: 484 ± 418 ml·kg^{-1}·min^{-1}, p <.05). There was a greater reduction in PC_r with BR during the 30% isometric contractions (1.6 ± 0.7 mM) compared to PLAC (3.9 ± 1.1 mM, p <.05) and showed consistency with their previous aerobic based research (Bailey et al., 2009) with BR lowering the $\dot{V}O_2$ slow component by 52% (BR: 100 ± 26 vs. PLAC: 209 ± 308 ml·kg^{-1}·min^{-1}). The authors suggest the reduced $\dot{V}O_2$ response following $N0_3^-$ supplementation was due to a reduced adenosine triphosphate (ATP) turnover in both 15% (BR: 192 ± 38 vs. PLAC: 296 ± 58 µM/s, p <.05) and 30% isometric contractions (BR: 436 ± 43 vs. PLAC: 607 ± 65 µM/s, p <.05). This subsequently allowed 30% isometric contractions to be tolerated for a greater time period in contrast to 15%. This emphasises, even with resistance exercise, there is consistency with other aerobic based research (Bond, Morton & Braakhuis,

2012; Hoon et al., 2014; Lansley et al., 2011b). A full summary of the studies in this literature review are presented in table 1.

Table 1: Summary of Research using Beetroot Juice for Exercise Performance

Author Group	Subject Number	Design	BR Dose and Duration	Exercise Protocol	Performance Outcome
Hoon et al. (2014)	10 highly trained male rowers	Placebo-controlled, double-blind crossover	70ml BR (4.2 mmol NO_3^-), 140ml BR (8.4 mmol	2000m rowing ergometer TT	70ml BR showed likely trivial effect on rowing

			NO_3^-) or PLAC (70ml NO_3^- depleted BR) two hours prior		ergometer TTC. 140ml BR showed a possible beneficial effect on rowing ergometer TTC
Muggeridge et al. (2013)	8 trained male kayakers	Randomised, double-blind crossover	70ml BR (5 mmol NO_3^-) or PLAC (70ml tomato juice) three hours prior	Kayak ergometer performance trials consisting of 15 min paddling at 60% maximum work rate, five 10s sprints and a 1km TT.	BR reduced steady state mean $\dot{V}O_2$ by 3.1%. BR had no effect on PO during all performance trials and 1km TTC
Bond, Morton & Braakhuis (2012)	14 well trained junior male rowers	Randomised, double-blind crossover	0.5L/day BR (5.5 mmol/day NO_3^-) or PLAC (0.5L/day blackcurrant juice) for six days	Six maximal 500m rowing ergometer intervals	Across all intervals, TTC in BR was likely improved vs. PLAC. TTC in intervals 1-3 revealed a possible negative effect of BR. Intervals 4-6 showed an almost certain benefit
Author Group	**Subject Number**	**Design**	**BR Dose and Duration**	**Exercise Protocol**	**Performance Outcome**
Cermak, Gibala & van Loon (2012)	12 trained male cyclists or triathletes	Double-blind, repeated-measures crossover	140ml BR/day (~8 mmol/day NO_3^-) or PLAC (140ml/day	60 min of submaximal cycling (2 × 30 min at 45% and 65% W_{max})	BR lowered submaximal $\dot{V}O_2$ by 3.5% at 45% W_{max} and 5.1% at 65% W_{max}. BR

			NO_3^- depleted BR) for six days	followed by 10km TT	improved 10km TT performance by 1.2% and mean PO by 2.1%
Christensen, Nyberg & Bangsbo (2013)	10 elite male cyclists	Placebo-controlled, randomised, double-blind crossover	0.5L of BR/day (6.2 mmol/day NO_3^-) or PLAC (0.5L/day blackcurrant juice) for six days	400 kcal cycling TT and six 20s sprints	BR had no effect on TT performance and PO during sprints
Lansley et al. (2011b)	Nine competitive male cyclists	Placebo-controlled, randomised, double-blind crossover	0.5L BR (6.2 mmol NO_3^-) or PLAC (0.5L NO_3^- depleted BR) two and a half hours prior	4km and 16km cycling TT	4km TT TTC and PO increased by 2.8% and 5% and 16km TT by 2.8% and 6% with BR. BR had no effect on $\dot{V}O_{2peak}$ during 4km and 16km TT
Cermak et al. (2012)	20 trained male cyclists	Double-blind, repeated-measures crossover design	140ml BR (8.7 mmol NO_3^-) or PLAC (140ml NO_3^- depleted BR) two and a half hours prior	One hour cycling TT	BR had no effect on TT TTC, PO or HR
Author Group	**Subject Number**	**Design**	**BR Dose and Duration**	**Exercise Protocol**	**Performance Outcome**
Wilkerson et al. (2012)	Eight well trained male cyclists	Randomised, double blind crossover	0.5L BR (6.2 mmol NO_3^-) or PLAC (0.5L NO_3^-	50 mile cycling TT	BR had no effect on TTC or PO. BR lowered mean

			depleted BR) two and a half hours prior		$\dot{V}O_2$ by 1.8% resulting in significant increase in PO/$\dot{V}O_2$ ratio by 3%
Larsen et al. (2007)	Nine well trained males	Randomised, double-blind, placebo controlled crossover	Sodium nitrate (0.1 mmol kg^{-1}/day) or PLAC (0.1 mmol kg^{-1}/day sodium chloride) for three days	Submaximal cycle ergometer work test	BR lowered submaximal $\dot{V}O_2$ resulting in the efficiency of cycling improving by 1.4%
Lansley et al. (2011a)	Nine physically active males	Randomised, double-blind crossover	0.5L/day BR (6.2 mmol/day NO_3^-) or PLAC (0.5L/day NO_3^- depleted BR) for six days	Submaximal and severe intensity bouts of "step" treadmill running	BR reduced the end $\dot{V}O_2$ cost by 7% in both submaximal and high intensity running
Kenjale et al. (2011)	Eight subjects with diagnosed PAD	Randomised, open-label crossover	0.5L BR (18.1 mmol NO_3^-) or PLAC (0.5L orange juice) three hours prior	Maximal treadmill CPX test	BR allowed subjects to walk 18% longer before claudication pain and maintained total peak walking time by 17%. BR lowered the fractional O_2 extraction of the gastrocnemius during the CPX
Author Group	**Subject Number**	**Design**	**BR Dose and Duration**	**Exercise Protocol**	**Performance Outcome**

Kelly et al. (2013)	Nine recreationally active males	Randomised, double-blind crossover	0.5L BR/day (8.2 mmol/day NO_3^-) or 0.5L/day PLAC (NO_3^- depleted BR) for a minimum of seven, and up to a maximum of 12 days	Four separate cycle ergometer ramp incremental tests	BR improved exercise tolerance by 17% at 60% and 12% at 80% peak power. 10% improvement at 100% peak power (not significant)
Bailey et al. (2009)	Eight healthy recreationally active males	Double-blind, placebo-controlled crossover	0.5L/day BR (5.5 mmol/day NO_3^-) or PLAC (0.5L/day blackcurrant cordial) for six days	Progressive "step" cycling protocols at moderate (80% GET) and severe intensity (70% difference between PO at GET and $\dot{V}O2_{peak}$)	BR reduced the primary amplitude of $\dot{V}O_2$ by 19% in moderate intensity and reduced the high intensity $\dot{V}O_2$ slow component by 23%
Wylie et al. (2013)	10 healthy males	Balanced crossover	140ml/day BR (8.4 mmol/day NO_3^-), 280ml/day BR (16.8 mmol/day NO_3^-) or equal volumes PLAC (NO_3^- depleted BR) for five days	Moderate intensity and severe intensity cycle exercise tests	140ml BR reduced submaximal $\dot{V}O_2$ by 2% and 280ml by 3%. 70ml BR had no effect

Author Group	Subject Number	Design	BR Dose and Duration	Exercise Protocol	Performance Outcome
Vanhatalo et al. (2010)	Eight healthy subjects	Balanced crossover	0.5L BR/day (5.2 mmol/day NO_3^-) or PLAC (0.5L/day blackcurrant cordial) for one, five or 15 days	Moderate intensity step test (90% GET) and incremental cycle ergometer ramp test	$\dot{V}O_{2max}$ and PO were greatest with 15 days of BR compared to day one and five and PLAC
Bailey et al. (2010)	Seven recreationally active males	Randomised, double-blind crossover	0.5L/day BR (5.1 mmol/day NO_3^-) or 0.5L/day PLAC (blackcurrant juice cordial) for six days	15% and 30% maximal voluntary isometric contraction "step" knee extension tests	BR lowered $\dot{V}O_2$ by 25% and PC_r during 15% isometric contractions BR lowered the $\dot{V}O_2$ slow component by 52% and PC_r during 30% isometric contractions

Conclusion

The purpose of this literature review was to explore research which has investigated the effects of BR on different measures of exercise performance. It is clear from the research reviewed that BR can be used as an ergogenic aid in recreationally active, trained and clinical subjects across varying exercise modalities. Performance outcomes from BR supplementation have been shown to reduce TTC and $\dot{V}O_2$ and increase PO and exercise tolerance, all of which have been demonstrated in well controlled study designs. The mechanisms responsible for these performance outcomes is through greater pools of bioavailable NO via the nitrate-nitrite-NO pathway which promotes increased blood flow

from the vasodilation of blood vessels and increased PO through a decrease in ATP turnover. However, not all research is in agreement, with these mixed findings potentially arising due to the respective training status of subjects, dose and duration of BR supplementation and the intensity and/or duration of the exercise protocols implemented. This can be shown by higher BR doses being required in trained populations due to baseline NO_3^- and NO_2^- levels being elevated, in addition to the greater development of the cardiovascular system. It also appears BR may be less effective during prolonged and less intense exercise due to NO responding more favourably in acidic environments.

Research is vast, particularly in trained populations, with this leading to a need for further research in recreationally active populations. In addition, the overwhelming majority of these studies have been conducted on males, both recreationally and trained, which opens up a requirement for specific female focused studies within the literature. Furthermore, aerobic based exercise has taken precedent in the literature which has left the investigation of the effects of BR on resistance based exercise an additional area in which current knowledge can be expanded.

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Research Article

Does an Acute, Single Dose of Beetroot Juice Decrease 1000m Rowing Ergometer Time to Completion in Recreationally Active Females.

Rationale for Proposed Journal

Medicine and Science in Sports and Exercise is a leading multidisciplinary original research journal. This journal features original investigations, clinical studies and comprehensive reviews on current topics in sports medicine and exercise science such as physical fitness, performance and physiology, sports medicine practice, epidemiology and biodynamics. The present study resembles performance and physiology within a current, relatively new area of research. Because of this, Medicine and Science in Sports and Exercise is the proposed journal of submission.

Abstract

Background and aims: Nitric oxide (NO) has gained considerable research interest for its ability to act as an ergogenic aid. The consumption of dietary nitrate (NO_3^-) has been shown to be a pathway to NO through the nitrate-nitrite-NO pathway. Beetroot juice (BR) has been shown to elicit favourable performance outcomes in a variety of exercise settings. The aim of the present study was to investigate whether an acute, single dose of BR would decrease 1000m rowing ergometer time to completion (TTC) in recreationally active females. **Methods:** 10 recreationally active females completed two 1000m rowing ergometer time trials (TT) following consumption of either 70ml of a commercially available BR or 70ml placebo (PLAC; tomato juice) two and a half hours prior in a randomized crossover design. **Results:** There was no significant difference in the TTC of the 1000m rowing ergometer TT (BR: 260.6 ± 47.5 vs. PLAC: 261.0 ± 50.8 s, *p* = .333) or in the mean heart rate (HR; BR: 162.5 ± 5.1 vs. PLAC: 160.1 ± 8.8 bpm, *p* = .151) and rating of perceived exertion (RPE; BR: 14.9 ± 1.0 vs. PLAC: 15.1 ± 1.2, *p* = .652). **Conclusion:** A single acute dose of BR appears to have no effect on 1000m rowing ergometer TTC in recreationally active females. Physiological adaptations to the cardiovascular system and the variability in baseline NO_3^- levels may partially account for these findings. Recreationally active populations may require greater doses of NO_3^- similar to that of trained populations to elicit an ergogenic effect.

Introduction

Nitric oxide (NO) has gained considerable research interest for its ability to act as an ergogenic aid (Petroczi & Naughton, 2010). Due to NO being both a free radical inorganic gas and cellular signalling molecule, it has been identified as the mediator in many physiological pathways including the increased blood supply and the lowering of blood pressure (BP) through the vasodilation of blood vessels (Förstermann & Sessa, 2012), increased glucose uptake (Tanaka et al., 2003), muscle contraction (Linden et al., 2011) and neurotransmission (Vincent, 2010). Because of these physiological responses to NO, there has been growing interest in the maintenance and enhancement of exercise through increasing the pools of bioavailable NO in the body (Bescós et al., 2012a).

Oxidation of the amino acid L-arginine, through the NO-synthase (NOS) enzyme family, is the classically thought of L-arginine-NO pathway which facilitates endogenous NO (Lundberg, Weitzberg & Gladwin, 2008). However, an alternative NOS independent pathway to NO, the nitrate-nitrite-NO pathway, was recently discovered whereby dietary inorganic nitrate (NO_3^-) can be reduced to nitrite (NO_2^-) and subsequently reduced to NO (Bailey et al., 2012; Lundberg & Govoni, 2004). Due to NO_3^- being readily available through dietary intake (Hord, Tang & Bryan, 2009), and without possessing the health risks linked with NO_2^- supplementation (Lundberg, Larsen & Weitzberg, 2011), increasing NO_3^- availability through dietary rich NO_3^- sources such as leafy green and root vegetables are emerging as an alternative method to improve exercise performance (Bailey et al., 2009; Larsen et al., 2007).

Beetroot juice (BR) has been shown to elicit favourable performance outcomes in a variety of exercise settings. One of the earliest reported BR studies, by Larsen et al.

(2007), demonstrated a 1.4% improvement in the efficiency of cycling (BR: 21.1 ± 1.3 vs. PLAC: 19.7 ± 1.6%, p <.01) through the reduction in oxygen consumption ($\dot{V}O_2$) from 2.98 to 2.82 L·min^{-1} (p <.02). This finding gained significant recognition for challenging the belief that exercise efficiency is resistant to any meaningful change, particularly since exercise efficiency among differing training levels was shown to be consistent (Moseley et al., 2004). Subsequent research has also shown beneficial effects of BR on exercise performance including the decreased amplitude of maximal oxygen consumption ($\dot{V}O_{2max}$) (Bailey et al., 2009; Lansley et al., 2011a; Muggeridge et al., 2013a; Wilkerson et al., 2012; Wylie et al.,2013), power output (PO) (Bender et al., 2018), increased exercise tolerance (Kelly et al., 2013; Kenjale et al., 2011), decreased time to completion (TTC) (Bond, Morton & Braakhuis, 2012; Hoon et al., 2014) or a combination of performance improvements (Bailey et al., 2010; Cermak, Gibala & van Loon., 2012; Lansley et al., 2011b; Masschelein et al., 2012; Vanhatalo et al., 2010). Of such studies, Lansley et al. (2011b), using an acute dose of BR (6.2 mmol of $N0_3^-$), reduced TTC and increased PO in a 4km and 16km cycling time trial (TT) (2.8% and 5%, and 2.7% and 6% respectively, all p <.01). In similar findings, Cermak, Gibala & van Loon (2012) also used an acute dose of BR (~8 mmol $N0_3^-$) and reduced TTC and increased PO during a 10km cycling TT (1.2% and 2.1% respectively). However, research has also shown no significant difference of BR on exercise performance (Arnold et al., 2015; Boorsma, Whitfield & Spriet, 2014; Cermak et al., 2012; Christensen, Nyberg & Bangsbo, 2013; Muggeridge et al., 2013b) and when using $N0_3^-$ salts (Bescós et al., 2012b; Peacock et al., 2012). Muggeridge et al. (2013b) showed no effect of an acute dose of BR (~5 mmol $N0_3^-$) on peak PO during a 10s sprint or 10km kayak ergometer TT.

The BR dose implemented in the literature has consisted mainly of chronic (Bailey et al., 2010; Bailey et al., 2009; Bond, Morton & Braakhuis, 2012; Boorsma, Whitfield & Spriet, 2014; Cermak, Gibala & van Loon, 2012; Christensen, Nyberg & Bangsbo, 2013; Kelly et al., 2013; Lansley et al., 2011a; Masschelein et al., 2012; Vanhatalo et al., 2010; Wilkerson et al., 2012) and acute, high volume protocols (0.5-0.75L) (Cermak et al., 2012; Kenjale et al., 2011; Lansley et al., 2011b; Vanhatalo et al., 2011). There is also less research investigating BR in recreationally active populations (Bailey et al., 2009; Kelly et al., 2013; Lansley et al., 2011a; Masschelein et al., 2012; Vanhatalo et al., 2010; Wylie et al., 2013). Of these, however, only Wylie et al. (2013) implemented a BR dose consisting of a concentrated, 70ml shot of BR which could be considered a more realistic, efficient option in a recreationally active setting.

Lower doses of NO_3^- have been theorised to be more effective among recreationally active compared to trained populations. This can be attributed by a blunted response to NO_3^- in trained populations arising from the cardiovascular system nearing physiological capacity (Bescós et al., 2012a) and baseline resting NO_3^- levels being elevated by 32% in comparison to untrained populations (45 ± 2 vs. 34 ± 2 μM, $p < .01$) as shown by Jungersten et al. (1997) and in agreement by Schena et al. (2002) who found an even greater elevation of 96% (49 ± 17 vs. 25 ± 2 μM, $p < .01$). Furthermore, to the best of knowledge, this is the first study to look at BR on 1000m rowing ergometer TTC in recreationally active females utilising a more realistic BR dose.

The rowing ergometer is a popular piece of exercise equipment in group fitness classes and gyms which requires consistent work of the whole body utilising both the aerobic,

and importantly the anaerobic energy systems at ~90% of $\dot{V}O_{2max}$ (Gillies & Bell, 2000; Hagerman, 1984). Crucially, this is an ideal environment for the reduction of NO_3^- to NO (via NO_2^-) which is heightened by acidic (Lundberg, Weitzberg & Gladwin, 2008; Modin et al., 2001) and hypoxic conditions (Castello et al., 2006; Cosby et al., 2003). Therefore, the aim of the present study was to investigate whether an acute, single dose of BR would decrease 1000m rowing ergometer TTC in recreationally active females.

Methods

Subjects

Ten recreationally active females, whose characteristics are presented in table 1, were recruited for the study from a local wellness centre. Two subjects did not comply with the study protocol and therefore were excluded. Subjects were classed as recreationally active if they did not compete at an elite level in any physically demanding sporting discipline, but consistently participated in the wellness centres fitness classes and reported being active at additional times during the week. Following an explanation of the study protocol and the associated risks which were presented on a participant information sheet (PIS; appendix 1) and upon completion of a physical activity readiness questionnaire (PAR-Q; appendix 2), written informed consent (appendix 3) was obtained from each subject. The study was approved by The University of Chester Faculty of Medicine, Dentistry and Life Sciences Research Ethics Committee (FREC; appendix 4).

Table 1: Subject Characteristics (N = 10)

Characteristic	**Mean ± SD**

Age (y)	34 ± 6.4
Body mass (kg)	65.6 ± 7.8
Stature (cm)	163.9 ± 4.0
Activity (h/wk)	5.1 ± 1.6

Research Design

Each subject was required to attend the studio on two separate occasions to complete a 1000m rowing ergometer TT. In a randomised crossover design, subjects were randomly assigned to consume either a 70ml commercially available BR (Beet-it, James White Drinks, Ashbocking, Suffolk) which contained a rich NO_3^- content (4.2 mmol NO_3^-), or a 70ml isocaloric placebo (PLAC; tomato juice [Schweppes, Coca Cola, London]) containing a negligible level of NO_3^-. The BR and PLAC were consumed two and a half hours before the commencement of each TT as this has been shown to be the duration in which plasma NO_2^- levels peak after consumption of NO_3^- (Kapil et al., 2010; Webb et al., 2008). Although it was not possible to blind the subjects, as each drink had a distinctive taste, the subjects were informed the purpose of the research was to investigate the effects of two different types of drinks on rowing ergometer performance and therefore were unaware of the true purpose of the study. Similar research protocols (Bailey et al., 2009; Bond, Morton & Braakhuis, 2012; Kenjale et al., 2011; Muggeridge et al., 2013; Vanhatalo et al., 2010) have been consistent in implementing this method of BR and PLAC delivery to their respective subjects. Both BR and PLAC were provided in the same bottle with no markings to prevent subjects reading

the manufacture statements in relation to the ergogenic effects of BR. To prevent influential bias, the lead researcher, who was present with the subjects during each TT, was not involved in the dissemination of the BR or PLAC. In addition, subjects were instructed to not reveal which drink they thought they may have been given to other subjects or the lead or assistant researcher.

Measurement Procedures

Indoor Row Time Trial

All rowing was completed using the Concept 2 Rower Ergometer (Model D, Vermont, USA) in a studio with no music and no other subjects or individuals present, with exception of the lead researcher. The TT were separated by a one week 'wash out period' with the time of day kept consistent (within two hours). Each subject had experience using the rowing ergometer which was essential in preventing an improvement relating to a learning effect which has been identified and controlled for in other research (Bond, Morton, and Braakhuis, 2012; Volianitis et al., 2001). Before the commencement of each TT, each subject was taken through an identical warm up led by the lead researcher consisting of dynamic stretching and three minutes of light intensity rowing. The PM5 performance monitor was set at 'single distance' setting of 1000m with subjects instructed to obtain the best time possible. The rowing ergometer flywheel was set at 'drag level 3' as recommended by the manufacturing company (Concept2 inc, 2018). Subjects were allowed to view the monitor which showed a basic display of metres rowed and stroke rate/min. Time elapsed and 500m pace average were covered to prevent subjects becoming motivated to improve their previous TTC.

The lead researcher had no further interaction with the subject until completion of the 1000m. Subjects completed a cool down of three minutes light intensity rowing following the TT.

Standardisation of Dietary Intake and Physical Activity

Dietary intake and physical activity was standardised for each subject using a variety of techniques (Jeacocke & Burke, 2010) to ensure there was no confounding effect on the BR and PLAC TT. Dietary intake was standardised by each subject recording their dietary and fluid intake on a 24 hour food diary (appendix 5) before the first TT with subjects instructed to replicate this 24 hours before the second TT. Prior to dietary recording, guidance was provided to each subject on how to accurately record dietary intake on a food diary. Subjects were not instructed to limit their consumption of foods containing high levels of NO_3^- so to replicate the most practical application of BR supplementation which has been consistent in other BR protocols (Boorsma, Whitfield & Spriet, 2014; Lansley et al., 2011a). Subjects were instructed, as also outlined in the PIS, to abstain from caffeine and alcohol 24 hours before each TT, and to preserve oral bacteria, which is known to facilitate the reduction of NO_3^- (Hezel & Weitzberg, 2013; Hyde et al., 2014), subjects were also instructed to abstain from mouthwash and chewing gum 24 hours before each TT as this has been demonstrated by Govoni et al. (2008) to attenuate the rise in plasma NO_2^- through abolishing oral bacteria by ~80%. Semi-quantitative analysis of each food diary, prior to the second TT, showed dietary intake was consistent for each subject. Physical activity was standardised for each subject through instructions to not

make any changes to their regular physical activity schedule, including exercise classes and recreational activity throughout the study duration.

Blood Pressure and Anthropometric Measurements

Upon arrival at the studio, two days before both TT, subjects had their BP and heart rate (HR) measured using The Microlife Automated Blood Pressure Device (Model #3AA1-2) to ensure an accurate baseline BP and HR measurement to compare against following both TT. This was due to the potential for BP and HR being elevated above baseline through the anticipatory response to exercise resulting from the stimulation of the sympathetic nervous system (Everson et al., 1996). Subjects had a period of seated rest for five minutes before four separate BP and HR measurements were taken, with the average of the last three measurements being recorded. The adherence to a set measurement protocol (O'Brien et al., 2005) was maintained for each subject. Proceeding baseline BP and HR, basic anthropometric measurements were taken (stature and body mass). Subject BP and HR measurements were repeated, following the same protocol, on the day of each TT upon arrival at the studio.

Heart Rate and Rating of Perceived Exertion

During both TT, average HR was measured using the Polar OH1 Optical Heart Rate Sensor (Kempele, Finland) and rating of perceived exertion (RPE; appendix 6) was measured every 250m using the Borg Scale (Borg, 1998) with an average RPE for each TT subsequently calculated. RPE has been shown to be positively associated with HR (Borg, 1970; Borg, 1982) which makes these two intensity measures a reliable and valid

combination for use in exercise protocols (Nelson et al., 2007; Roemmich et al., 2006; Whaley et al., 1997). Each subject was educated on the importance of the correct and accurate use of the RPE scale prior to each TT (Borg, 1998; Whaley et al., 1997).

Data Analysis

Data is reported as median and range (median ± range) for TT TTC as the data violated the assumption of normal distribution. Data is reported as mean and standard deviation (SD; mean ± SD) for all other analysis. Differences between BR and PLAC TTC were analysed using a Wilcoxon signed-rank test and average HR and RPE were analysed using a paired samples t-test both of which had an alpha set at $p \leq .05$. Differences between baseline, PLAC and BR systolic and diastolic BP and resting HR were analysed using a one-way repeated measures analysis of variance (ANOVA). Power analysis was conducted using G*Power (Erdfelder, Faul & Buchner, 1996) to determine a sufficient sample size assuming an alpha of $p \leq .05$, a power of .80, a large effect size ($dz = 0.8$) and two tails (Faul et al., 2009). Based on these assumptions, the desired sample size was set at 15. All statistical procedures were completed using SPSS statistics version 24 for windows.

Results

On observation of the TTC in both BR and PLAC TT conditions it was apparent there was no significant difference in performance (Table 2).

Table 2: Summary of Time Trial Results

Condition		TTC (s)	Average TT HR (bpm)	RPE	Resting SBP (mmHg)	Resting DBP (mmHg)	Resting HR (bpm)
BR	Mean	260.6*	162.5	14.9	114.6	67.3	67.7
	± SD	47.5*	5.1	1.0	11.1	5.8	6.0
	p value	.333	.151	.652	.957	.970	.355
PLAC	Mean	261.0*	160.1	15.1	114.2	67.6	70.3
	± SD	50.8*	8.8	1.2	8.3	3.9	7.4
	p value	.333	.151	.652	.957	.970	.355
Baseline	Mean	_	_	_	114.1	67.4	67.9
	± SD	_	_	_	8.3	3.8	4.7
	p value	_	_	_	.957	.970	.355

Note: *=median ± range

1000m Time-Trial Performance

There was no significant difference in the TTC of the 1000m rowing ergometer TT between BR (260.6 ± 47.5 s) and PLAC (261.0 ± 50.8 s, *p* = .333; Figure 1). There was no significant difference in the mean HR between BR (162.5 ± 5.1 bpm) and PLAC (160.1 ±

8.8 bpm, *p* = .151; Figure 2) and no significant difference in RPE between BR (14.9 ± 1.0) and PLAC (15.1 ± 1.2, *p* = .652; Figure 3).

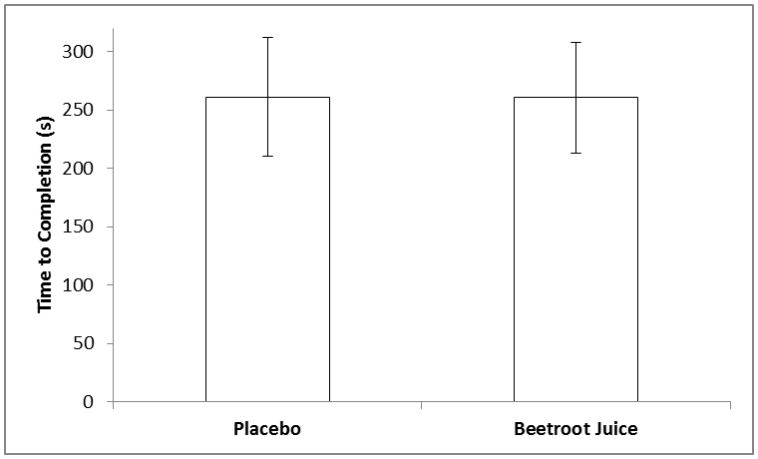

Figure 1: 1000m rowing ergometer time to completion following 70ml beetroot juice or placebo, median ± range, *p* = .333.

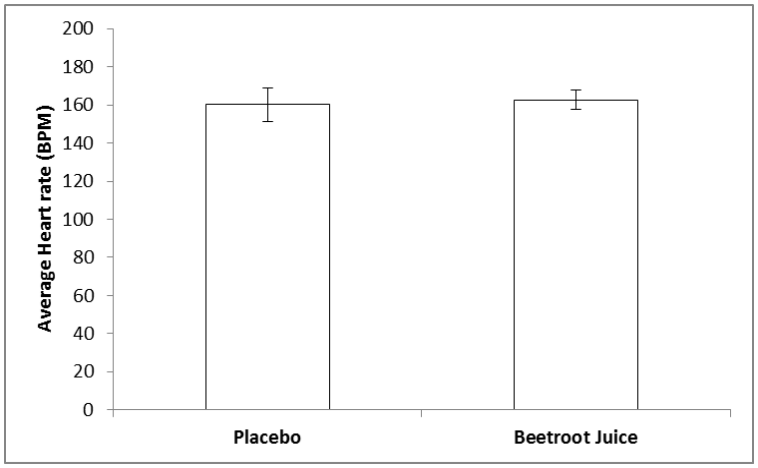

Figure 2: 1000m rowing ergometer mean heart rate following 70ml beetroot juice or placebo, mean ± SD, *p* = .151

Figure 3: 1000m rowing ergometer mean rating of perceived exertion following 70ml beetroot juice or placebo, mean ± SD, p = .652.

Resting Blood Pressure and Heart Rate

There was no significant difference in resting systolic BP between baseline (114.1 ± 8.3 mmHg), BR (114.6 ± 11.1 mmHg) and PLAC (114.2 ± 8.3 mmHg, p = .957) and was consistent with resting diastolic BP between baseline (67.4 ± 3.8 mmHg), BR (67.3 ± 5.8 mmHg) and PLAC conditions (67.6 ± 3.9 mmHg, p = .970; Figure 4). There was no significant difference in resting HR between baseline (67.9 ± 4.7 bpm), BR (67.7 ± 6.0 bpm) and PLAC (70.3 ± 7.4 bpm, p = .355; Figure 5).

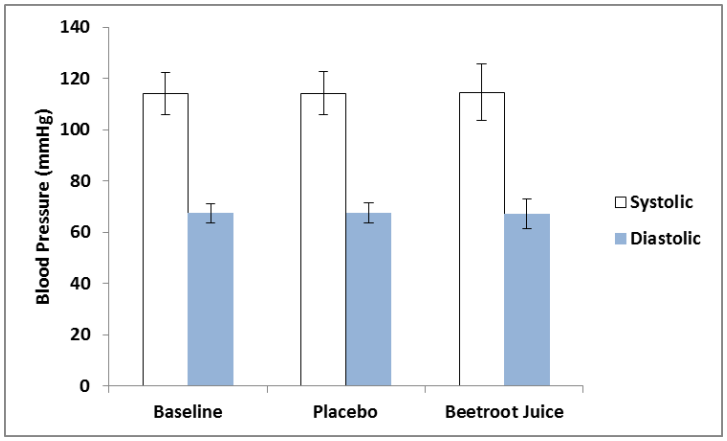

Figure 4: Systolic and diastolic blood pressure at baseline, following 70ml beetroot juice and placebo, mean ± SD, *p* =.957, *p* =.970.

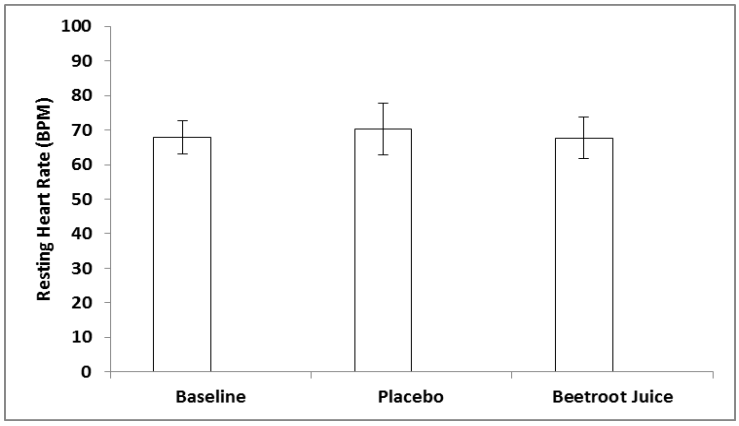

Figure 5: Resting heart rate at baseline, following 70ml beetroot juice and placebo, mean ± SD, *p* = .355.

Discussion

The principle finding from this study demonstrates that a single 70ml dose of BR did not improve 1000m rowing ergometer TTC in recreationally active females. Furthermore, additional findings were also consistent showing no difference in resting systolic and diastolic BP, resting HR and mean TT HR and RPE. This is in contrast with previous aerobic based research, in which the focus was also specifically on recreationally active populations whereby BR elicited an improvement in respective exercise performance (Bailey et al., 2010; Kelly et al., 2013; Kenjale et al., 2011; Lansley et al., 2011a; Vanhatalo et al., 2010). However, the present study findings are in agreement with Wylie et al.

(2013) who still found exercise performance improvements with BR but only attained this response with a chronic, greater dose of NO_3^- than implemented in the present study (8.4 mmol/day NO_3^- and 16.8 mmol/day NO_3^- for five days). Interestingly, Wylie et al. (2013) was unable to achieve exercise performance improvements with BR when using the lowest BR dose in their respective protocols, consisting of 70ml BR (4.2 mmol NO_3^-) which is identical to the NO_3^- dose used in the present study. Moreover, rather than using an acute dose, a chronic dose of five days was used which still yielded no significant difference in the performance measurements of end exercise $\dot{V}O_2$ (BR: ~1.61 ± 0.21 vs PLAC: 1.64 ± 0.21 L·min^{-1}), time to task failure (BR: ~510 ± 30 vs. PLAC: ~470 ± 27 s) and blood lactate (BR: 1.2 ± 0.5 vs. PLAC: 1.2 ± 0.2 mM, all p >.05). Furthermore, there is a distinct difference in the application of BR in the present study, consisting of an acute single dose of 70ml BR (4.2 mmol NO_3^-), in comparison to the research which has shown consistent improvements with BR on recreationally active populations in aerobic based exercise (Bailey et al., 2009; Kelly et al., 2013; Lansley et al., 2011a; Vanhatalo et al., 2010). A more effective strategy for BR supplementation has been suggested to be chronic dose protocols (Hoon et al., 2014) which is consistent in all of the aforementioned studies who implemented chronic doses of six (Bailey et al., 2009; Lansley et al., 2011a), seven to 12 (Kelly et al., 2013) and a combination of one, five and 15 days BR dose duration (Vanhatalo et al., 2010). Of such studies Vanhatalo et al. (2010) showed that 15 days of BR supplementation improved $\dot{V}O_{2max}$ (BR: 3.50 ± 0.82 vs. PLAC: 3.42 ± 0.88 L·min^{-1}) and PO (BR: 331 ± 68 vs. PLAC: 323 ± 68 W) greater compared with one day of BR supplementation (3.42 ± 0.82 L·min^{-1} and 325 ± 71 W, both p <.05) and Bailey et al. (2009) who demonstrated that six days of BR ingestion reduced the primary

amplitude of $\dot{V}O_2$ by 19% in moderate intensity cycling (PLAC: 640 ± 146 vs. BR: 521 ± 153 ml·min^{-1}, p <.01) and the $\dot{V}O_2$ slow component in high intensity cycling was reduced by 23% (PLAC: 739 ± 242 vs. BR: 568 ± 195 ml·min^{-1}, p < .05). The chronic application of BR on the respective recreationally active subjects was clearly sufficient to elicit favourable exercise performance outcomes. Furthermore, chronic dose protocols over several days may induce alterations in mitochondrial protein expression, which is unlikely to be seen after an acute dose of BR. This was investigated by Larsen et al. (2011) who showed respiratory control ratio (RCR) of skeletal muscle was higher after $N0_3^-$ ingestion (8.5 ± 0.7) compared to PLAC (6.5 ± 0.7, p =.06) and also increased the phosphate/oxygen (P/O) ratio with $N0_3^-$ by 19% (1.36 ± 0.06 to 1.62 ± 0.07, p = .02).

However, it can be argued that the practicality for recreationally active populations to adopt this method of chronic BR supplementation in order to achieve any meaningful exercise performance outcomes is not realistic. The present study was based around a realistic protocol which could be adopted with ease on a more consistent basis. Because of this, the BR protocol was set at an acute dose in contrast to the chronic dose protocols used by the aforementioned studies. An exception to this was Kenjale et al. (2011) who demonstrated that subjects with diagnosed peripheral artery disease (PAD) were able to walk 18% longer before claudication of pain and maintained total peak walking time by 17% with acute BR supplementation. The drastic difference in the BR protocol by Kenjale et al. (2011) was the remarkably high 18.1 mmol $N0_3^-$ three hours prior to exercise which is four times greater than a typical daily dietary intake consisting of 50-140mg $N0_3^-$ (Mensinga, Speijers & Meulenbelt, 2003). Even though the 1000m rowing

ergometer TT can be viewed as a shorter, more anaerobic form of exercise (Gillies & Bell, 2000; Hagerman, 1984), which has been shown to potentiate the reduction of NO_3^- to NO (via NO_2^-) (Lundberg, Weitzberg & Gladwin, 2008; Modin et al., 2001), the acute dose of 4.2 mmol NO_3^- in the present study was clearly not sufficient to elevate NO through the nitrate-nitrite-NO pathway to levels which have been shown to increase the vasodilation of blood vessels regulating blood flow (Förstermann & Sessa, 2012), muscle contractility (Linden et al., 2011), glucose uptake (Tanaka et al., 2003) and neurotransmission (Vincent, 2010) all contributing to the improvement in exercise performance. In light of this, it might require an even greater hypoxic and acidic physiological environment, created by a shorter, even more intense exercise protocol to elicit an exercise improvement. This can be seen in the study by Bond, Morton and Braakhuis (2012) who implemented six 500m sprints using the rowing ergometer and found that across all intervals, TTC in BR was likely improved compared with PLAC (0.4, 95% CI ± 1.0%). More importantly, intervals 4-6 showed an almost certain benefit (1.7, 95% CI ± 1.0%) which suggests the increasing demands on the body, generating greater acidity, proved to elicit a NO response through the nitrate-nitrite-NO pathway which subsequently improved exercise performance. However, has mentioned previously, the improvement may have been due to the chronic BR dose used (5.5 mmol NO_3^- for six days) in comparison to the present study.

Another possible theory responsible for the negligible exercise performance in the present study is the training status of the recreationally active subjects. Although the subjects were not classed as elite or non-elite athletes, they were involved in consistent

recreational activity which involved periods of high intensity exercise and may be responsible for the negligible effect of BR. This may be due to a greater adaptation of the cardiovascular system (Bescós et al., 2012a) and individual variability in baseline resting NO_3^- and NO_2^- levels ranging from 32-96% greater in comparison to individuals who are sedentary or partake in low levels of physical activity (Jungersten et al., 1997; Schena et al., 2002) who could be expected to respond more favourably to BR.

It can also be argued that not limiting the subject's consumption of NO_3^- rich foods prior to the TT could have been a prominent factor in the negligible outcome of the results as plasma NO_3^- levels may have already been elevated. However, to preserve ecological validity, it was deemed appropriate to instruct the subjects to continue on their normal dietary intake to represent the most valid form of BR supplementation with this method being undertaken in previous research (Boorsma, Whitfield & Spriet, 2014; Lansley et al., 2011a; Lansley et al., 2011b; Peacock et al., 2012). The BR dose did not affect systolic or diastolic BP and may be attributed to the low NO_3^- dose used. Although studies have been consistent in showing BR supplementation lowers both systolic and diastolic BP (Bailey et al., 2010; Larsen et al., 2007; Webb et al., 2008) through the vasodilation of blood vessels from NO elevation (Förstermann & Sessa, 2012) this was not the case in the present study. The primary reason for this is the low dose of BR used and can be supported by the recommendation of the dietary approach to prevent hypertension by Appel et al. (1997) in which a daily intake of 20 mmol NO_3^- is advised (Hord, Tang & Bryan, 2009), representing more than three times that of the present study.

Limitations

The present study was not without limitation, arising from sampling difficulties and limited resources which affected the variety in methods being utilised in the data collection. The desired sample size of 15, determined by power analysis using G*Power (Erdfelder, Faul & Buchner, 1996) to achieve a power of .80, was not possible due to a limited availability of subjects which ultimately reduced the power of the study. However, the sample size may be viewed as sufficient according to Lansley et al. (2011b), who in their respective research calculated that a sample size of nine resulted in a .80 power to enable a 2-3% difference in endurance TT at an alpha of .05.

The limited resources available resulted in the possibility of only a select number of tests being undertaken. Namely, analysis of the concentrations of plasma NO_3^- and NO_2^- would have been of significant benefit to determine whether the BR dose had an effect on NO production as the combination of plasma NO_3^- and NO_2^- levels are commonly used in determining levels of NO (Moncada & Higgs, 1991). Controversially, the reliability of this method for assessing plasma NO has been questioned (Lauer et al., 2001) due to a number of studies contradicting the current paradigm that demonstrates NO_3^- and NO_2^- are useful indicators of endogenous NO concentrations and that plasma NO_3^- levels can be influenced by a number of independent factors such as the short half-life of NO_2^- (Kelm, 1999; Vallance et al., 1995). In addition, the inability to measure $\dot{V}O_2$ responses in the BR and PLAC TT conditions limited the data analysis. Although there was no difference in the TTC in the present study results, there may have been an altered $\dot{V}O_2$ response demonstrating a possible greater efficiency during the TT as shown in other BR research (Bailey et al., 2009; Bailey et al., 2010; Cermak, Gibala & van Loon, 2012;

Lansley et al., 2011a; Larsen et al., 2007; Muggeridge et al., 2013b; Wilkerson et al., 2012; Wylie et al., 2013).

Further limitations are evident in the self-reporting of dietary intake via the 24 hour food diary. Although each subject reported being consistent with their nutritional intake 24 hours prior to each TT, it is not possible to rule out inconsistencies in the nutritional intake of each subject which would have affected the performance of each TT due to differences primarily in total energy and carbohydrate intake (ACSM, 2009). This is evident in the study by Lansley et al. (2011b) in which TT were rescheduled four times due to poor dietary adherence from the subjects, but only if this was mentioned by the subjects.

Conclusion

To the best of knowledge this is the first study to investigate the ingestion of a single, acute dose of BR and its effects on rowing ergometer TT performance in recreationally active females. The primary finding from this study demonstrates that an acute, single dose of 70ml BR (4.2 mmol $N0_3^-$) two and a half hours prior to exercise was not sufficient in improving 1000m TT performance in recreationally active females. Recreationally active populations may require greater doses of $N0_3^-$ similar to that of trained populations. The research was challenged due to various limitations, from the low sample size and the inability to measure plasma $N0_3^-$ and $\dot{V}O_2$ responses in the BR and PLAC TT conditions arising from limited resources. A greater sample size, along with these additional measurement procedures should be included in further research which

is required on sedentary and less active populations, relative to the present study, investigating the optimal BR dose required to elicit an ergogenic effect.

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